

## A COMPARISON OF MITOCHONDRIA IN PLANT AND ANIMAL CELLS.<sup>1</sup>

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The recent discovery, through the elaboration of new methods of technique, of the wide distribution of mitochondria has attracted the attention of many investigators in widely separate fields. Their characteristic form, strongly suggestive of bacteria, is now quite familiar. The technique was devised by anatomists and was applied by them to plant cells. I refer particularly to Meves' ('04, p. 284) work on *Nymphæa*. But, as might have been expected, these structures did not altogether escape the careful scrutiny of the older botanists, despite their imperfect methods of technique; for Zimmermann ('93, p. 215) certainly observed and described mitochondria in the living hair cells of *Momordica elaterium* and in the meristem and root tip of *Vicia faba*. Nevertheless botanists in this country have been slow to study mitochondria notwithstanding the fact that in properly made preparations they constitute a cell organ as conspicuous as the nucleus.

Unhappily we have deplorably little experimental evidence to lead us to any conclusion as to their functional significance in the cell economy. Our curiosity has been so insistent that we have resorted to less reliable sources of information, one of which is the argument from analogy. It is said that since the mitochondria are found in almost all active cells, their function must be a generalized one which they all possess in common. The validity of this line of reasoning rests entirely upon the resemblance of mitochondria in these different cells. Mitochondria, however, differ slightly in their solubility in acetic acid and in other respects, and in direct proportion to the extent of variation the above argument loses force.

<sup>1</sup> This work was also carried on at the Marine Biological Laboratory, Woods Hole, Mass., where the Director, Dr. Lillie, very kindly placed a research room at my disposal. It was aided by the Carnegie Institution of Washington.

A very important issue at stake is the relationship of plant and animal mitochondria. Are the researches of the botanist of interest to the anatomist and vice versa? Should we attempt to coördinate and correlate the two, and, if so, how far can we go? Though these questions have never been directly attacked, considerable difference of opinion is apparent in the literature. Suffice it to say that Pensa ('14, p. 22) states that the mitochondria in plant and animal cells are not identical; Guilliermond ('13*a*, p. 481) and Smirnow ('06, p. 153) think that they are; Duesberg and Hoven ('10, p. 99) believe them to be homologous; while Sapěhin ('15, p. 320) holds an intermediate view. He thinks that in the higher plants mitochondria-like forms may be divided into two groups: "plastids" and "chondriosomes" which, however, are indistinguishable in the early meristem.

I have devoted my whole attention to this single problem of the relationship of plant and animal mitochondria with the hope of obtaining results which are clear-cut, concise and definite, bringing to bear upon it old, as well as entirely new, methods of technique in the form of supravital dyes of the janus green series which have never before been applied to plant cells.

#### OBSERVATIONS.

The statements concerning the similarity or the dissimilarity of plant and animal mitochondria have been made, with but few notable exceptions, by investigators having personal experi-



FIGS. 1 and 2. Cells from the pea and the pancreas fixed in formalin and bichromate, mordanted in bichromate (Regaud IV. B) and stained with iron hema toxylin. (1,500 diameters.)

ence with one or the other, seldom with both. It is essential that the two should be compared side by side.

I have chosen for my experiments the cells of the radicle of the pea and the acinus cells of the pancreas of the mouse on account of the close resemblance of their mitochondria. The general appearance may be seen by reference to Figs. 1 and 2. Morphologically the mitochondria would seem to be identical in the two; but in reality this is not the case, for I have examined them very carefully with a high magnification (3,500 diameters) and I find that they are, on the whole, slightly longer and thicker

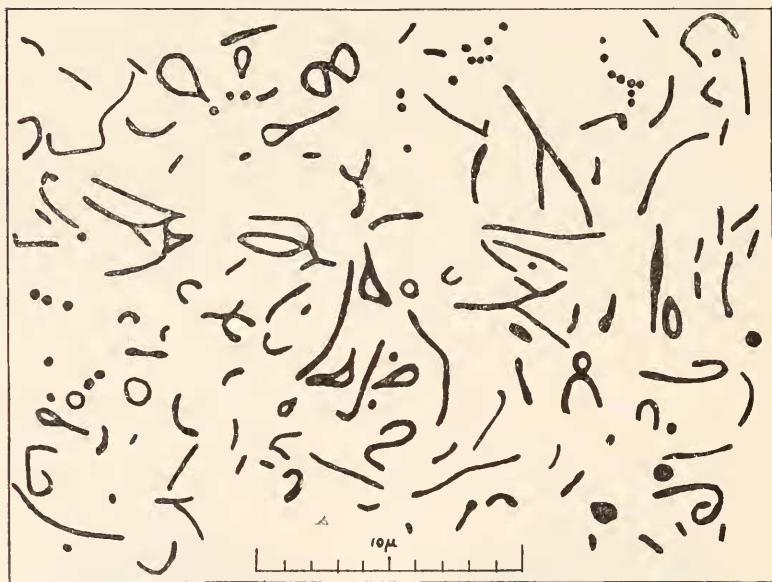


FIG. 3 Selected mitochondria from cells of pea radicle prepared by Regaud IVB to show the extent and limits of the polymorphism. (3,500 diameters.)

in the pancreas. I have made detailed drawings to scale of selected mitochondria from both. This can be done with considerable accuracy by making use of a good camera lucida, with appropriate illumination, and a sharply pointed pencil. The results are illustrated in Figs. 3 and 4. Filaments, rods and granules predominate. Branching filaments, networks, spherules and so on are rarer, but occur in both. In fact no form, however bizarre, is to be found in the one for which a counterpart cannot

be discovered in the other. The absence of very minute mitochondria, merging into the invisible, is of interest from the point of view of an origin *de novo* through condensation; and the polymorphism, in both the pea and the pancreas, makes plain the hopelessness of any attempt to devise a system of individual nomenclature, based upon morphology, to embrace all the forms. Obviously the material selected permits of very close comparison.

I have taken precautions in the following experiments to eliminate, as far as possible, chance variations due to incurrent

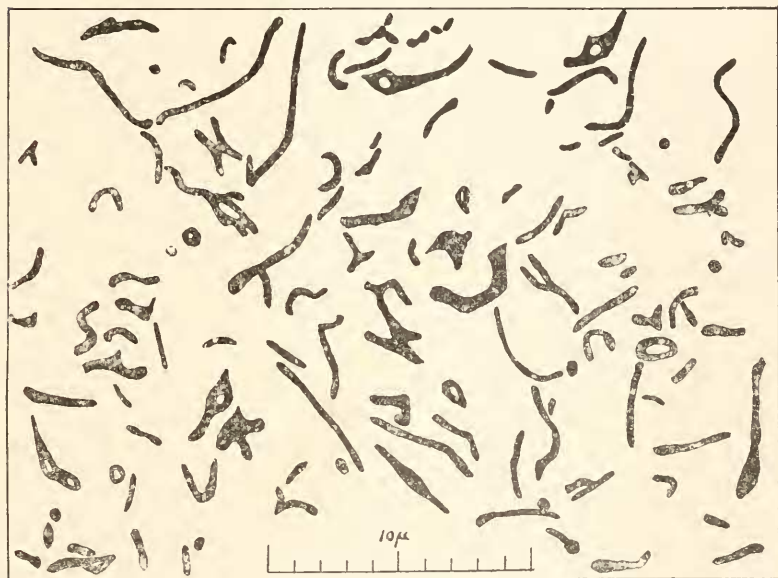


FIG. 4. Selected mitochondria from acinus cells of the mouse, treated in exactly the same way, showing similar polymorphism. (3,500 diameters.)

experimental errors which would, however, in any event, be likely to effect both tissues similarly (see p. 207). They were fixed together in the same bottle, dehydrated together, cleared together and embedded in the same block of paraffin together. They were cut with the same sweep of the knife, mounted on the same slide and stained at the same time. Many preparations have been made and in each of them the dehydration, clearing and embedding were carried on in the same way, so that the results are comparable. The sections were stained with iron



hematoxylin, with fuchsin methyl green, with Altmann's fuchsin picric acid and sometimes by the Benda method. The chief results are briefly set forth in the following table, but a large number of additional controls were made.

In a general way, running our eyes down the table, we see that the response of mitochondria to fixatives is the same in plants as in animals. Similar fixations preserve them, modify them and destroy them in much the same way in both. It also shows that the technique is easy, not difficult, and that a number of chemicals, which are generally thought to be destructive, will fix them more or less satisfactorily.

The standard mitochondrial fixatives, like Regaud ('10, p. 296) II., III., IV. *A* and IV. *B*, Benda's modification of Flemming's fluid, the acetic acid osmic bichromate mixture of Bensley, and Zenker, without acetic, will fix them in both tissues. I have found, however, that Regaud's mixtures are by far the most satisfactory because they do not produce the artificial coagulation of the ground substance caused by the others. They also preserve the true form of the mitochondria more faithfully; though I have reason to suspect that in some cases, in plant cells, they cause fragmentation and shrinkage. I have studied the behavior of the ingredients of these fluids.

Formalin is perhaps the most important since it penetrates rapidly, retains the homogeneous appearance of the ground substance and preserves the mitochondria satisfactorily. Formalin alone (Sapěhin, '15, p. 321) serves as an excellent fixative for mitochondria. My best results were obtained with a solution of formalin of from 5 to 10 per cent. made up from ordinary commercial formalin neutralized with magnesium carbonate. More dilute solutions tend to cause a swelling of the mitochondria and more concentrated ones a shrinkage; but different tissues require different concentrations. Regaud advises a subsequent mordanting with bichromate, but I find that this is quite unnecessary. Used in combination with 3 per cent. potassium bichromate, according to the directions of Regaud, formalin gives the best of fixations with both the pea and the pancreas, though in my experience the results may be even improved by diluting the mixture with an equal volume of water as advised

TABLE I.<sup>1</sup>

No.	Fixative.	Radicle of Pea.	Pancreatic Acini.
1615B..	5% formalin 41 days	+normal	+normal
1574A..	5% " 4 "	+normal	+normal
1575A..	10% " 4 "	+normal	+normal
1616A..	40% " 4 "	+irregular, shrunken	+nodular, shrunken
1580....	2.5% " 4 " mordanted in 3% bi- chromate for 21 days	+but slightly swollen	+swollen and frag- mented
1574....	5% "	+normal	+normal
1575....	10% "	+fragmented	+few
1576....	20% "	+normal	+few
1616B..	40% "	+normal	+normal
1585....	formalin 10 c.c., satu- rated aq. picric acid 40 c.c. (Regaud II) 1 day	+fragmented	+normal, indistinct.
1585B..	same, mordanted in 3% bichromate 20 days (Regaud III)	+normal, unusually filamentous	+normal, indistinct
1577A..	5% formalin, 10 c.c., sat. aq. picric acid 40 c.c., 1 day	+fragmented, distorted	+fragmented
1577....	same, mordanted in 3% bichromate for 16 days	+fragmented, distorted	—
1578....	10% formalin, 15 c.c., 1% chromic, 85 c.c.; mordanted in pyroli- gneous 1 day and 2% bichromate 1 day	+fragmented	+few and fragmented
1566....	formalin 20 c.c., 3% bi- chromate, 80 c.c.; mordanted in 3% bi- chromate 15 days. (Regaud IVB)	+normal	+normal
1628...	Formalin 10 c.c., sat. — aq. corrosive subli- mate 40 c.c. 1 day	—	—
1629....	Formalin 10 c.c., sat. aq. — corrosive sublimate 20 c.c., sat. aq. picric acid 20 c.c. 1 day	—	—
1582....	Boiling water 5 min.	—	—
1583....	95% alcohol	—	—
1447....	Gradual increase in con- centration of alcohol, 2.5, 5, 7.5, 10, 15, 20, 30, 40, 50, 70, 80, 95% and absolute; chloro- form	—	—
1584....	95% alcohol 10 c.c., 3% bichromate 10 c.c. 4 days	—	—
1618....	2% osmic 2 c.c. 2.5% bi- chromate 8 c.c., acetic 1 drop	+indistinct	+normal
1609....	sat. alcohol sol. corro-? sive sublimate	?	—

"+" signifies present, "—" absent and "?" doubtful.

TABLE I—*Continued.*

No.	Fixative.	Radicle of Pea.	Pancreatic Acini.
1610....	sat. aq. corrosive sublimate	+distinct, shrunken	+few, indistinct
1611A..	sat. aq. picric acid	+nearly normal	+few, fragmented
1612....	2% chromic acid	+somewhat distorted	+few, fragmented
1613....	3% bichromate	+normal	+nodular rods, rings, granules
1614....	.5% osmic acid	+unsatisfactory	+normal

by Sapěhin ('15, p. 321). Formalin in combination with picric acid is a somewhat erratic fixative, very excellent in some instances and bad in others. In good preparations the mitochondria appear very large and not so shrunken as when acted on by other fixatives. Formalin and chromic acid constitute a poor fixative which fragments the mitochondria and, in some cases, destroys them entirely.

Potassium bichromate used alone (Sapěhin, '15, p. 321) is a good preservative for mitochondria but penetrates badly. Regaud recommends its use in mixtures and as a mordant in order to render the mitochondria insoluble in the alcohols during dehydration. The bichromate which remains in the tissue when it is sectioned, increases its affinity for fuchsin when the fuchsin methyl green stain is applied.

Ethyl alcohol in 95 per cent. solution destroys the mitochondria in both the pea radicle and the pancreas. When alcohol is employed in gradually increasing concentration the same result is obtained. When it is combined with potassium bichromate the mitochondria are also destroyed. In fact our conclusion can be none other than that alcohol, in whatever combination, is a very poor fixative for mitochondria.

Boiling water preserves the general form relations but does not bring to light any mitochondria.

Picric acid is a fairly good fixative for mitochondria in the cells of the pea and pancreas, when used in a saturated aqueous solution. It has the great advantage that it penetrates well.

Chromic acid alone causes considerable distortion of the mitochondria and somewhat irregular coagulation of the ground substance but in combination it is apparently very efficacious as shown by its inclusion in Benda's modification of Flemming's fluid and in other mixtures.

Osmic acid is the best preservative of mitochondria and the worst penetrator and is therefore useless in the cells of the pea radicle on account of their impermeable cellulose walls; yet it is this quality of being an excellent preservative which makes it so valuable in association with other chemicals. It does not cause any artificial coagulation of the ground substance.

Corrosive sublimate in saturated aqueous solution also penetrates the pea radicle very poorly; the pancreas not so badly. However it preserves the mitochondria in the pea far better than in the pancreas in spite of the fact that they are, in both cases, somewhat shrunken. In alcoholic solution it is destructive, but it is difficult to tell whether this is due to the alcohol or to the increased amount of sublimate. In Zenker's fluid the injurious action of the sublimate is counter-balanced by the presence of potassium bichromate.

The action of acetic acid is so important that I have made a detailed study of it as set forth in Table II.

In the first place I have varied the concentration of acetic acid in Regaud's mixture IV. *B* from 0 per cent. to 10 per cent. With no acetic acid the mitochondria are normal; while with a concentration of 2.5 per cent., or more, they are destroyed in both tissues, showing that their degree of solubility is the same.

I have also experimented with Zenker's fluid and I find that, with it, the mitochondria are on the whole much more resistant to acetic acid. This may be due to the fact that the Zenker's fluid was only applied for one day, instead of for five days like the Regaud's fluid; or the presence of sublimate in the Zenker's fluid may have tended to counteract the action of the acetic acid. The mitochondria are fairly well preserved when no acetic acid has been added to the Zenker's fluid. With increase in acetic acid, up to 10 per cent., they become destroyed in the deeper parts of the tissue, especially in the pea; while they are fairly well preserved in the most superficial cells in both. In the pea, also, the mitochondria are far more prone to become fragmented. This difference between surface and interior seems to be a question of penetration rather than of a difference in the mitochondria themselves. There is a simultaneous action of all the ingredients of the fixative on the cells at the surface and here the

TABLE II.

No.	Fixative.	Radicle of Pea.	Pancreatic Acini.
1570....	Zenker 0% acetic 1 day	+better preserved in older cells, apt to be vesicular, fragmented	+normal
1571....	" 2.5% "	+better preserved in older cells, apt to be vesicular, fragmented	+normal in peripheral cells, absent in center of tissue
1572....	" 5% "	+almost all destroyed in younger cells, still fairly numerous in older ones	+fewer, only in peripheral cells slightly fragmented
1573....	" 10% "	+more fragmented and vesicular	+same
1617....	" 20% "	+fairly well shown in all cells	+slightly fragmented, present in all cells
1627....	" 33% "	+but very rare	+but very rare
1626....	" 50% "	—	—
1566....	Regaud IVB, 0% acetic 5 days, mordanted in 3% bichromate 8 days	+normal	+normal
1567....	Regaud 2.5% acetic 5 day	—	—
1568....	" 5% "	—	—
1569....	" 10% "	—	—
1625....	Equal parts acetic and water	—	—
1624....	Pure acetic	—	—
1565....	Benda no acetic 8 days	+rather vesicular	+normal
1579....	Benda, 0.8% acetic, mordanted in formalin 20 c.c.; 3% bichromate 80 c.c. 6 days	+normal few	+normal
1579B..	Same but mordanted in pyroligneous 1 day and 2% bichromate 2 days	+normal few	+normal

bichromate, and possibly also the sublimate, modify the action of the acetic acid. In the deeper layers of the tissue, on the other hand, we have to do with a successive action of the said ingredients, determined by their relative rates of penetration. The acetic acid penetrates first and acts upon the mitochondria before the bichromate and the sublimate have come upon the scene.

Mitochondria are surprisingly resistant to higher concentrations. They are well preserved by a mixture of Zenker's fluid and 20 per cent. acetic acid and their relations are shown in Figs. 5 and 6. With 33 per cent. acetic acid only a few scattered mitochondria remain, while they are completely destroyed by a mixture containing 50 per cent. of acetic acid.

Pure acetic acid in half concentration and in full concentration destroys the mitochondria in both tissues. But it must not be thought that acetic acid in small concentrations is to be avoided because I have found that Benda's fluid without acetic acid does not seem to act so well as with acetic acid, in the amount prescribed.

In other words acetic acid exercises a solvent action on mitochondria which is the same in plants as in animals and varies with the distance of the cells from the surface and with the



FIGS. 5 and 6. Cells from the pea and the pancreas fixed in Zenker's fluid containing 20 per cent. of acetic acid and stained with fuchsin and methyl green. (1,500 diameters.)

character of the other components of the fixative. There is reason to believe, further, that the resistance of mitochondria in certain young plant cells is less than in old ones, calling to mind the condition which obtains in the spermatogenesis of many animals where the mitochondria in the young cells are more susceptible to acetic acid than in the mature spermatozoa (Regaud, '08, p. 661); which comparison makes the resemblance of plant and animal mitochondria, in this respect, still closer.

I have attempted to ascertain the experimental error in the study of mitochondria in fixed tissues in plants and animals and to see whether it is the same in both.

The conditions of the experiment are not so important as the nature of the fixative but they must nevertheless be taken into consideration. Variations in the temperature (under say 46° C.) and the illumination of the tissue prior to fixation do not seem to influence the mitochondria for I have grown peas at room



temperature and in the refrigerator (8 to 12° C.); in bright light and in darkness without bringing about any noticeable change in their mitochondria. Some fixatives are rather unstable and cannot be kept, even for a few hours, in bright sunlight or in a warm place. Under these conditions Regaud's formalin and bichromate mixture, for example, undergoes a rapid change characterized by a darkening in color, but its action does not seem to be impaired, though, to be on the safe side, one should avoid it. On the other hand, mechanical manipulation of the tissue before fixation often causes very confusing alterations in the mitochondria especially in the softer animal tissues.

The distribution of mitochondria within the cell is not altered appreciably in either plants or animals by the technique used, except in instances where mitochondria are present with different solubility, some being preserved and others being destroyed. This is of common occurrence in the pea radicle where with some fixatives, filaments only appear; the granules being obliterated so that the apparent distribution is altered.

The technique makes a very great difference in the number of mitochondria in the preparation. Some ingredients of the fixative are particularly likely to destroy them, like the acetic acid already mentioned; so that in the study of unfamiliar tissues we must assure ourselves that the technique is adapted to show all the mitochondria present.

The shape of mitochondria is so easily modified by the fixative that we must be on our guard here also, especially in plant cells. That is to say, we frequently meet with a fragmentation of mitochondrial filaments, the filaments of the living cell appearing in the form of rows of granules in the fixed preparation. The reverse change never takes place, for filaments are never formed through a coalescence of granules, under the influence of the fixative. Poor penetration of the fixative in the deeper layers of the tissue often causes the mitochondria to lose their characteristic form and to swell up into large spherules or vesicles. Similarly if a film on the surface of the tissue is allowed to dry, before fixation, the mitochondria in it will be profoundly modified.

The size of mitochondria is also subject to some modification, as shown by the comparison of mitochondria in living cells with

those in fixed tissues. The general tendency of fixatives is to shrink them and to make them uniformly smaller than they really are in the living condition. This tendency is very slight, almost negligible, in the case of the best fixatives. On the other hand, acetic acid, and possibly also formalin in very low concentrations, have a marked swelling effect.

It must be admitted that variations sometimes do occur in the mitochondria in our preparations, particularly in the pea, for which no reasonable explanation can be advanced on the basis of the technique. They may result from rhythmical variations in the activity of the cells which we have reason to believe occur, just like periodicity in cell division, many cells dividing almost synchronously (Kellicott, '04, p. 531) but they cannot be wholly explained in this way. They may result also from the conditions under which the radicle is growing, upon whether it is submerged or exposed to the air; but I find that radicles grown under exactly the same conditions occasionally show mitochondrial variations which are very perplexing.

Evidently the experimental error is something which we must have considerable respect for, but which can be controlled if the proper precautions are taken. Its identity in plant and animal tissues is another indication of the similarity of the mitochondria in the two.

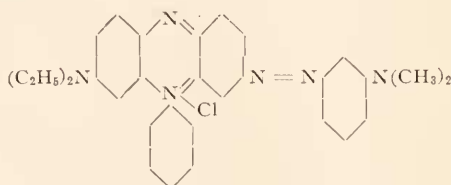
There is another factor, often very confusing, which we have to bear in mind, namely that of the production of coagula in the homogeneous ground substance. They are formed in the protoplasm of the pea radicle by exposure for about 30 minutes to a temperature of 48° C. or higher and also by fixatives containing acetic acid, chromic acid, corrosive sublimate or alcohol. Iron hematoxylin, when imperfectly differentiated with iron alum solution, stains these coagula in the same manner as the mitochondria and in many cases no clearly marked distinction can be made between them, since they both vary very much in their resistance to differentiation. The formation of these coagula is generally accompanied by distortion, fragmentation or destruction of the mitochondria. In the cells of the pancreas these fixatives bring about a distinctly fibrous appearance in the normally homogeneous basophilic substance which is often as re-

sistant to differentiation as are the mitochondria, but the destructive effect is not so apparent as in the cells of the pea radicle.

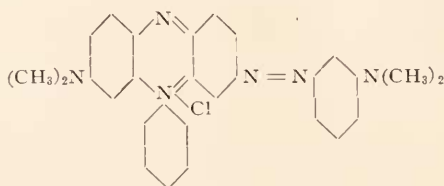
The question of the reactions of plant and animal mitochondria, after fixation, to stains may be dismissed with very few words. When they have been fixed by any method, it is usually possible to color them with any stain, by the Benda method, with fuchsin-methyl-green, with fuchsin-picric acid or with iron hematoxylin, but it is sometimes advisable to select some mordant in order to induce the stain to take. The Benda method is the most difficult and it is sometimes refractory, for no apparent reason, in tissues where the others give excellent results. In other words there is nothing whatsoever specific about the staining reaction.

A number of supravital stains have been discovered for mitochondria in animal cells, of varying degrees of specificity, and it is interesting to note that none of them have heretofore been applied to plant mitochondria. The most delicate is the janus green reaction, which we owe primarily to Michaelis ('99, p. 565). The delicacy of this reaction is shown by E. V. Cowdry's ('16, p. 429) observation that janus green B will stain mitochondria specifically in human lymphocytes in a dilution of half a million (*i. e.*, one part of stain to 500,000 parts of salt solution), and that janus green G will not stain them even in a relatively strong solution.

Janus green B is diethylsafraninazodimethylanilin:



Janus green G is dimethylsafraninazodimethylanilin:



Janus green G differs only in the substitution of a *dimethyl* group in the place of the *diethyl* group in the safranin molecule. Janus blue and janus black I were first introduced as vital stains for mitochondria by E. V. Cowdry ('16, p. 431). Janus blue is the trade name for diethylsafranin-B-naphthol and janus black I is a mixture of diethylsafraninazodimethylanilin and some other dye of unknown constitution. Diethylsafranin may easily be made from janus green by splitting off the azodimethylanilin group (Cowdry, '14, p. 269). I have applied these dyes to the mitochondria in the cells of the pea radicle and the acinus cells of the pancreas with the following results.

TABLE III.

Dye.	Cells of Pea Radicle.	Acinus Cells of Pancreas.
Janus green B. ....	+ Intense	+ Intense
Janus green G. ....	—	—
Janus blue. ....	+	+
Janus black I. ....	+	+
Diethylsafranin. ....	?	? + faint
Nilblue B, extra. ....	+ faint	+ faint

It will be seen that the mitochondria in plant cells react to vital stains in precisely the same way as in animal cells, even to this extraordinarily delicate janus green test. It is very much more difficult, however, to get a good stain in plant cells by reason of their tough, cellulose walls offering an almost insurmountable barrier against the penetration of the dye; and janus green is at best but a poor penetrator compared with neutral red, methylene blue and others. For this reason the staining of mitochondria is much slower than in animal cells. Great difficulty was also experienced in finding a suitable medium for the examination of the two tissues. At first they were examined, side by side, in weak aqueous solutions of the dyes to which sodium chloride had been added in varying amounts. This proved entirely satisfactory for the pancreas, but failed with the pea, for which a sugar solution was used. Another trouble with the pea, not encountered with the pancreas, is that the cells are cemented closely together and are therefore so very difficult to separate by teasing, that thin sections of the living pea radicle had to be cut with a razor blade. This method also is objection-

able because a comparatively large amount of the tissue juice is liberated which brings about a troublesome precipitate of the dye and makes it necessary to renew it frequently. In short, plant cells are very hard to handle but the stain is none the less specific. The petals of some flowers (narcissus, sweet pea, freesia, etc.) give very much more satisfactory results than the pea since with them the dyes penetrate more easily.

I have also tried to stain the living plants *in toto* by growing them in sand moistened with a strong solution of janus green B. I discovered at once that they react differently to this treatment. Peas were stunted, but grew and flowered without any coloration with the exception of the root hairs and small portions of the epidermis which seemed to be dead. On the other hand, gourds grew vigorously for a time and became intensely stained while growing. They presented a very beautiful appearance because in some of the tissues the dye is reduced to its color base, diethylsafranin, so that the plant is marked off into red and bluish green areas following the distribution of processes of reduction and of oxidation. Cells from the different regions were examined and it was found that the whole protoplasm was stained more or less uniformly and that the mitochondria were not specifically colored. These experiments would seem to indicate that the vital staining of plants with the less toxic azo dyes is full of promise from the point of view of plant physiology.

This specific comparison of the mitochondria in the pea and in the pancreas has shown that morphologically they are almost identical, save for a slight difference in diameter. No forms are present in the one for which counterparts cannot be discovered in the other. If they could be viewed dissociated from their environment it would be next to impossible to tell which belonged to the pea and which to the pancreas. Microchemically they are identical so far as our imperfect methods go. Similar fixatives preserve them, modify them and destroy them in like fashion in both. Even the experimental error is the same. Finally, and most important of all, they react the same way to the janus green test and to other supravital dyes. Accordingly our provisional conclusion can be none other than that the mitochondria in the pea and in the pancreas are composed of precisely the same material.

But this is only a single isolated instance and many facts of interest can be brought to light by a broad general discussion of mitochondria in many forms which I shall now venture to make, based in part upon the literature.

## DISCUSSION.

### *Distribution.*

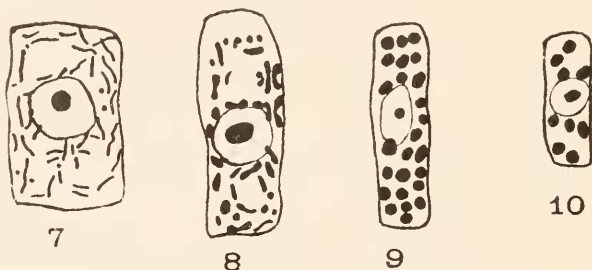
In plants mitochondria have been recorded from the *Angiosperms* to the *Fungi*; but it is difficult or even impossible to demonstrate their existence in the very lowest forms of plant life, like the *Myxomycetes*, the *Schizomycetes* and some of the *Algæ*; though in these groups structures of questionable nature have been discovered which may ultimately prove to be mitochondrial. This absence of typical mitochondria in the lowest plants may be contrasted with their almost universal occurrence in the *Protozoa*. In all forms of animals, from amœba to man, which have been investigated with adequate methods of technique, they occur without exception.

With regard to the different types of cells. In plants, they occur from the tip of the root to the end of the stem, wherever the protoplasm is active, with but few exceptions. The same is true in the various categories of animal cells. They are met with in gland cells, nerve cells and muscle cells; in connective tissue cells, germ cells and almost all others, except in the terminal stages of cytomorphosis. And this is one of the greatest points of similarity between these granulations in the plant and animal kingdoms, that it to say their progressive diminution and final absence in the later stages in the life of the cell.

I refer, for instance, to the decrease in number of mitochondria in plant cells, which runs parallel to the formation of chloroplasts, for it is said (Guilliermond, '12, plates 17-18) that when the plastids are fully mature few if any mitochondria remain (which reminds one of the single large chloroplast and the absence of mitochondria in some algæ); and these are mature and highly differentiated cells. In animals there is a similar disappearance of mitochondria in the life cycle of red blood cells. In the young, nucleated forms they are very abundant, they become less and less so as the cell differentiates; a few persist after the nucleus is



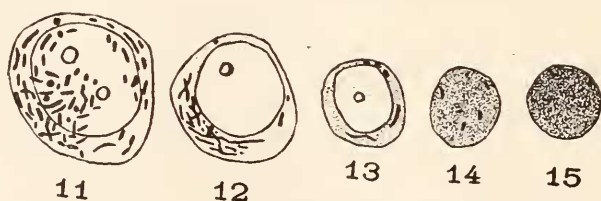
lost; but in the fully mature forms, as they occur in the circulation in man, mitochondria are entirely absent. In plants this disappearance is associated with the production of chlorophyll; in animals, with the formation of hemoglobin, two substances with strikingly similar chemical constitution; in both it is progressive and runs parallel with an increase in the degree of



FIGS. 7, 8, 9 and 10. Meristem and parenchyma cells of the bean (after Guillemont, '12, plate 18, modified) showing the progressive disappearance of mitochondria with the formation of plastids containing chlorophyll.

differentiation and with the age of the cell, general metabolism being diminished and special functions being accentuated (Figs. 7-15).

This diminution of mitochondria in cytomorphosis is really of very common occurrence but it attracts attention only in those



FIGS. 11, 12, 13, 14 and 15. Erythroblast, megaloblast, normoblast and erythrocytes from bone marrow of a rabbit stained vitally with janus green showing the parallelism between the disappearance of mitochondria and the appearance of hemoglobin in the form of a diffuse deposit. (1,500 diameters.)

cells which normally die and are replaced in large numbers, collectively, in the life of the organism.

In the separate regions of the cell, also, there is a general similarity in the distribution of mitochondria in plants and animals. Where the cells are elongated, as in the plerome,

filamentous mitochondria are usually distributed parallel to the long axis; which calls to mind the arrangement of mitochondria in gland cells, nerve cells, muscle cells and others. In the serous cells of the parotid they are heaped up in the proximal region next the basement membrane and remote from the lumen (Fig. 16). Where the polarity is reversed as in the thyroid (Fig. 17), the mitochondria are also reversed; while in the epithelial cells of the intestine, they are condensed at both poles (Fig. 18). This, Champy ('12, p. 109) thinks, is indicative of a double polarization for adsorption and for secretion. Corresponding condensations have not been recorded in plant cells.

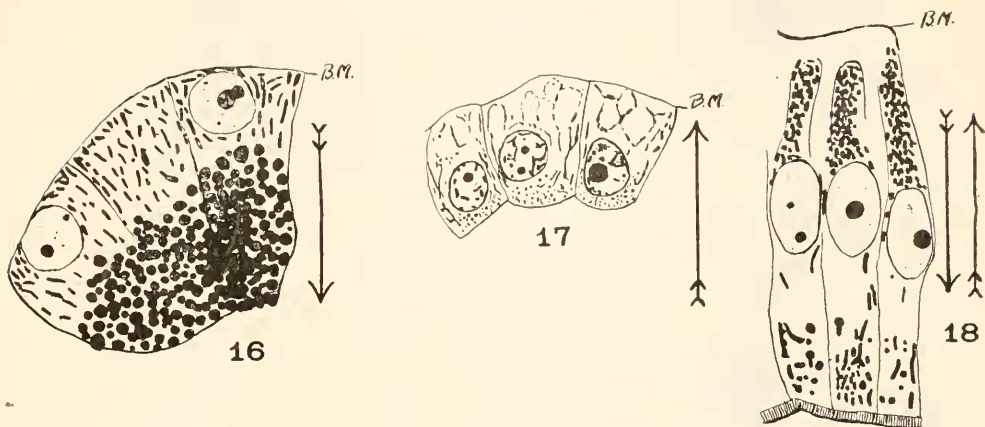


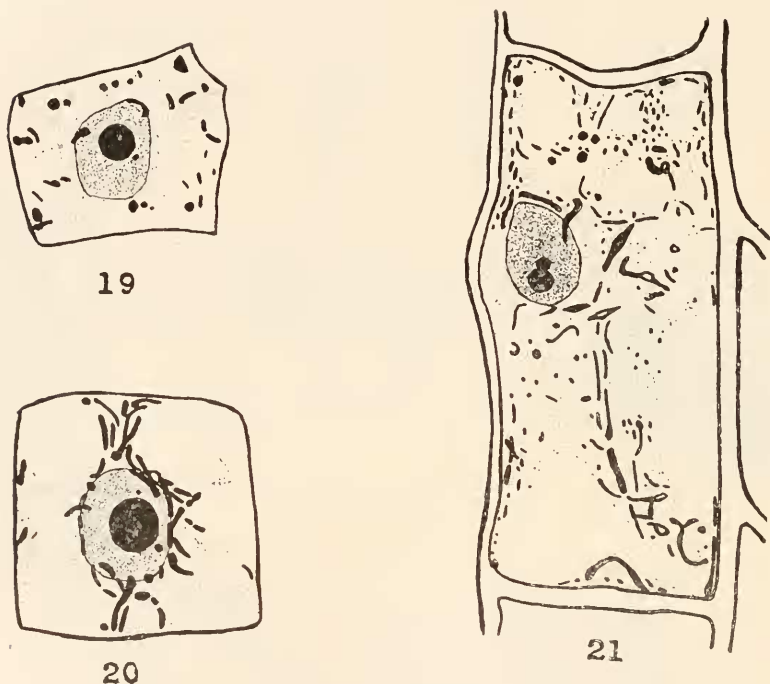
FIG. 16. Serous cells of parotid of mouse with proximo-distal polarity, as represented by the arrow, B. M. being the basement membrane. (1,500 diameters.)

FIG. 17. Thyroid cells (after Bensley, '16, p. 55) with reversed polarity, disto-proximal, in the direction of the basement membrane.

FIG. 18. Intestinal epithelial cells with double polarity, mitochondria being accumulated at both poles. (1,500 diameters.)

Mitochondria, however, group themselves about the nucleus in both plants and animals. In the early meristem of plants, generally, mitochondria are found indifferently distributed in the protoplasm (Fig. 19). They soon approach and appear to come into actual contact with the nucleus (Fig. 20) in which position they enlarge and form plastis which migrate away from the nucleus and become distributed more or less evenly in the surrounding protoplasm (Fig. 21). Guilliermond has repeatedly described this migration. I find that the mitochondria become

progressively more resistant to acetic acid in this process of plast formation. Similarly in the spermatogonia of certain animals the mitochondria are diffusely arranged (Fig. 22); in the spermatocytes they approach the nucleus (Fig. 23) and become so closely applied to it that investigators have been deluded into thinking that they actually originate from it. In the later stages of spermatogenesis they leave the nucleus (Fig. 24)



FIGS. 19, 20 and 21. Meristem and young and old cortical cells of the pea fixed in formalin and picric acid (Regaud III) and stained with iron hematoxylin showing primary diffuse arrangement of mitochondria, secondary condensation about the nucleus and final even distribution throughout the whole cytoplasm. (1,500 diameters.)

becoming more resistant to acetic acid as Regaud ('08, p. 662) has shown. Indeed the parallelism is very close. Other instances might be cited.

In plant cells one rarely finds a condensation of mitochondria in the peripheral cytoplasm though it is frequently met with in animal cells. It is particularly manifest in certain stages in the

growth and maturation of the egg as Van der Stricht ('09, plate 1) and his pupils, among others, have abundantly shown. The causes underlying these strange movements of mitochondria are obscure.

Furthermore, during cell division the mitochondria are distributed in much the same way in plants as in animals. They persist during the whole process; they are absent in the spindle area, whether a definite spindle be formed or not; and they are divided in approximately equal amounts between the two daughter cells.



FIGS. 22, 23 and 24. Spermatogonium, spermatocyte and spermatid from mouse testis fixed in formalin and bichromate (Regaud IVB) and stained with iron hematoxylin. Note diffuse arrangement of mitochondria, circumnuclear condensation and final diffuse arrangement. (1,500 diameters.)

In animal cells they are almost invariably disposed in a radial fashion about the centrosome: contrasting strongly with their appearance in *Pustularia* during spore formation when they are entirely absent from the region near the centrosome and are found in a clump in the portion of the cells farthest away from it (Guilliermond, '13*b*, p. 649). No examples of a radial arrangement have to the best of my knowledge been described in the higher plants but this may be due to the well-known absence of a typical centrosome in the *Angiosperms*.

It will be noticed that in animal cells rather more variations obtain in the arrangement of mitochondria than in plant cells, but this seems to be correlated in some way with the fact that animal cells are more generally polarized. I mean polarized for irritability, conduction, secretion, contraction and so forth, properties which do not play so great a rôle in the life of plants, where separate regions of the cell are not so distinctly marked off. Accordingly if one were searching for variations in the distribu-

tion of mitochondria in different parts of plant cells one would be inclined to examine cells polarized with reference to light, secretion and so on, where cytoplasmic division of labor may be expected.

### *Morphology.*

As we pass down the plant scale we find no noteworthy differences in the morphology of mitochondria, even in those *Algæ* which possess them they are alike. Similarly, few animals have mitochondria of distinctive morphology, though in certain species of both plants and animals, either filamentous or granular forms may predominate.

In the different tissues of plants there is some variation in the size and shape of mitochondria. In some cells thick filaments are most abundant, while in other kinds of cells, granular forms of variable size may be found. Networks are rarely met with. The question is complicated by the fact that in certain tissues all morphological transitions between typical mitochondria and true plastids are to be seen; swellings develop in the region of the mitochondrial filaments, which apparently grow larger and larger and ultimately form mature plastids.

In the different tissues of animals there is rather more variation in the morphology of mitochondria. They are usually filamentous in gland cells, rod-like in muscle cells and they are often granular in egg cells. Networks occasionally occur in the pancreas and in spermatogonia as well as in other locations. Even within gland cells of the zymogenic type there is considerable variation. The mitochondria in the acinus cells of the pancreas are uniformly longer and thicker than those in the chief cells of the fundus of the stomach and I find that they are two or three times as long as those in the serous cells of the parotid of the mouse (compare Figs. 2 and 16). The mitochondria in zymogenic cells often possess little enlargements which are supposed to be the precursors of granules of zymogen, and which call to mind the swellings on the mitochondria in plant cells during plastid formation. The similarity of the process may be seen by taking two specific instances; the production of metachromatic corpuscles in plants as figured by

Guilliermond ('13c, p. 438) and the formation of fat as described by Dubreuil ('13, p. 104B). Compare Figs. 25 and 26.

Striking differences also obtain in the morphology of mitochondria in the different categories of nerve cells. Nicholson ('16, p. 347) has found that they are usually filamentous, especially in the anterior horn cells and in the cells of the reticular formation; they are rod-like and granular in the large and small cells of the Gasserian ganglion, and they occur in the form of large irregular blocks in the cells of the trapezoid nucleus.

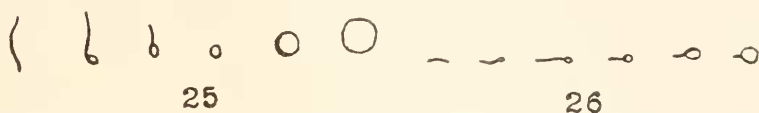


FIG. 25. Metachromatic corpuscles developing in mitochondrial filaments (after Guilliermond, '13c, p. 438).

FIG. 26. Droplets of fat forming in mitochondrial filaments (after Dubreuil, '13, p. 104).

The greater polymorphism of animal cells, with regard to their mitochondrial content, probably results from the fact that their functions are more diversified, for they have a greater variety of duties to perform under different conditions. Apparently their distribution within the cell is more varied in animals than in plants for the same reason (p. 212). The form of mitochondria is apparently independent of the degree of mobility of the surrounding protoplasm.

It is worthy of note that in developing tissues of both plants and animals similar and parallel changes take place in the morphology of mitochondria, at least in certain instances. In the spores of plants and the egg cells of animals, they are usually granular and sometimes filamentous; in the developing tissues of the embryo they are usually filamentous and rarely if ever granular. With the assumption of special functions on the part of the cells in the different organs they change their form. Some remain filamentous and acquire blebs (secreting cells), others become more rod-like (muscle), still others granular, and so on.

A single cell may contain all the types of mitochondria which have been enumerated. This is true for animals as well as for



plants. Generally, however, filamentous, rod-like, dumb-bell and granular forms are met with. Filaments, when present, have an astonishingly uniform diameter in the same cell, though they may possess the swellings already mentioned. Segmentation of filaments is of common occurrence in both and it is possible that a coalescence of granules may take place. In some tissues there is considerable variation from cell to cell, while in others the morphology of the mitochondria is quite uniform throughout. Thus the "anthocyane" producing mitochondria, figured by Guilliermond ('13*a*, p. 479), are uniform; those in the cortical cells of the pea radicle, variable in length. In neighboring acinus cells of the pancreas of the mouse they are uniform; in contiguous spinal ganglion cells, variable.

A fundamental distinction may be made between variations in length and variations in breadth, in the case of both plant and animal mitochondria, to which I have seen no reference in the literature. While the mitochondria in the cells of the pea radicle vary greatly in length, filamentous and rod-like forms predominating, their girth is remarkably uniform, throughout the whole tissue, in individual cells and in different parts of the same filament (Fig. 1). The same holds for the pancreas (Fig. 2). The uniformity in girth extends right to the end of the filaments which always end abruptly without tapering. The truth of this statement is made still more apparent by examining any illustration of mitochondria, because artists are wont, quite rightly, to draw each filament with a single sweep of the same pen or brush. The one great exception to this rule is plastid formation in plants and the production of bleb-like swellings in association with secretion in animal cells. The diameter of the mitochondrial filament is fixed, but the length is not; one is stamped on the cell through its organization and is probably dependent on the water content of the surrounding cytoplasm, the other is most likely an expression of growth by accretion affecting the length but not the diameter. We have here two attributes, independently variable, which may perhaps be influenced in different ways, common to both plant and animal mitochondria, which speak more strongly for their identity than any other.

*Composition.*

We must bear in mind the unsatisfactory nature of the evidence upon which our views of the chemical constitution of mitochondria are based. It is impossible to make a direct chemical analysis of them. We are forced to use solvents, special stains and so forth. We know astonishingly little positively, but we infer a great deal, often on very insufficient grounds. Most of the work has been done with animal cells, and investigators have arrived at the general conclusion that here mitochondria are of the nature of phosphatids, in other words, that they contain fatty acid, phosphoric acid, glycerol and some nitrogen base. Lecithin is an example of a typical phosphatid and has been used to manufacture artificial mitochondria. Briefly the evidence is as follows:

1. In animal cells it has been found that mitochondria are almost completely soluble in alcohol, ether, chloroform and dilute acetic acid. They are rendered insoluble by chromization, and also, in my experience, by treatment with formalin, at least in some cases. They do not stain with Sudan III. or Scharlach R. They are only sometimes blackened with osmic acid.

In plant cells they are also soluble in alcohol, ether, chloroform and dilute acetic acid and they are here rendered insoluble by chromization in exactly the same way. Treatment with formalin also makes the mitochondria insoluble. The whole cell blackens with osmic acid so readily that it is impossible to ascertain just how strongly the mitochondria themselves react to it.

2. It is said, in the literature, that part of the mitochondrial substance, in animal cells, is not soluble in these fat solvents and it has been assumed that this portion is albumin. The results of applying Millon's reagent are uncertain and difficult to interpret.

In view of the statement of Forbes and Keith ('14, p. 73) that vegetable phosphatids differ from those in animal cells in that they nearly always contain sugar firmly bound to the rest of the molecule, we should bear in mind the possibility of sugar being a likely constituent of mitochondria in plants.

3. Artificial mitochondria have been made by Löwschin ('13, p. 203) of lecithin and albumin solutions which apparently

present the same form and solubilities as true mitochondria in both plant and animal cells. They form granules, rods and filaments which multiply by division. He embedded them in glycerin-gelatin, fixed them and found that they stained in the usual way by the various mitochondrial methods.

I have myself experimented with lecithin. I made a very fine emulsion, evaporated it to dryness on glass slides and stained with iron hematoxylin. The bodies which I observed resembled mitochondria in some respects but in others differed so very radically from them that I am unable to confirm Löwschin's observations.

4. The temperature solubility of mitochondria may be also significant because mitochondria, like phosphatids, are thought to have a low dissolving point. Policard ('12, p. 229) observed, in the case of some animal tissues, that the mitochondria are dissolved when subjected to a temperature of from 48° C. to 50° C. in a moist atmosphere from 10 to 30 minutes, while the other parts of the cells remain practically unaffected; and Koch and Voegtlin ('16, p. 59) remark that phosphatids are notoriously unstable to heat. With the hope that this temperature solubility would turn out to be a definite physical test for mitochondria, I experimented with both the pea and the pancreas, observing the precautions outlined on page 199. I found that in both the pea and the pancreas they dissolve at about the same temperature, that is to say, from 48° to 50° C., when treated for 30 minutes. The first change noted is a loss of their filamentous form (see also the Lewises, '15, p. 375); they became granular with indistinct outlines merging into the surrounding cytoplasm and they finally disappeared leaving no trace behind. In the case of the pea, the mitochondrial changes are difficult to make out on account of the production of a confusing coagulation of the ground substance.

5. The specific gravity of mitochondria is generally greater than that of the protoplasm in which they are embedded (Fauré-Fremiet, '13, p. 602). This is determined by the unsatisfactory centrifuge method. When they are thrown down they are said to be of high specific gravity. If the protoplasm is in the physical condition of a gel, rather than a sol, as in the nerve cell, the dis-

tribution of mitochondria is unaltered by centrifuging, as Key discovered. But this is no reason to believe that these mitochondria differ from others in their specific gravity. At any rate, where the method is applicable (*i. e.*, in egg cells) the mitochondria are heavier than protoplasm, in which respect they conform to what we know of phosphatids and differ sharply from oils and neutral fats which rise to the surface and float, instead of being thrown down.

6. In some animal cells mitochondria act as solutes for different substances. Pigments of various kinds are frequently found dissolved in their substance so that they assume the most brilliant hues. Asvadourova ('13, p. 263), following Prenant, calls them "chromochondria" on this account. The presence of droplets of neutral fat within the mitochondrial filaments has also been recorded.

Plant mitochondria behave in exactly the same way, anthocyanin, chlorophyll and other pigments, sugar, starch and even fat being heaped up within them (Guilliermond, '13*b*, p. 647).

7. There seems to be a certain correspondence between variations in the histological picture of mitochondria and variations in the phospholipin content of the same organ on chemical analysis. Thus Mayer, Rathery and Schaeffer ('14, p. 612) have been able to alter the mitochondria experimentally in liver cells. In stages with more mitochondrial substance, chemical analyses show an increase in phosphorized lipid; in stages with less, diminution.

Unfortunately mitochondria in plant cells have not been investigated from this point of view.

8. And, finally, the very interesting observations of Russo and Rene Van der Stricht must be mentioned. Russo ('12, p. 215) claims to have been able to increase the number of mitochondria in the oöcytes of the fowl by the injection of lecithin and Rene Van der Stricht ('11, p. 435) obtained results which seemed to be confirmatory.

Since there have been no observations along this line in plant cells I have attacked the problem by growing peas in solutions of lecithin. The results were in no sense definite and concise, though the mitochondria did seem to be increased in diameter.

*Physiology.*

It is generally conceded that mitochondria in plant cells play an important part in the elaboration of chlorophyll and starch. They are thought to do this through the intermediary of the chloroplasts. According to Guilliermond ('12, p. 387) chlorophyll appears in typical mitochondria, increases in amount, other changes take place and the mature chloroplast results. It is not surprising, therefore, that mitochondria have attracted so much attention among European botanists, because the formation of these substances had been under discussion for nearly a century and a deadlock had been reached before these new mitochondrial methods were devised. Indeed the formation indirectly of starch from atmospheric carbon dioxide and water, through the action of sunlight on chlorophyll, is the most fundamental of all vital processes in plants. Mitochondria are concerned in the formation of chlorophyll and thus the very existence of the plant depends on them. Plants furnish the food of animals so that the importance of mitochondria with respect to the food supply is apparent.

Very obviously the mitochondria in typical animal cells can take part in no such process since there are no plastids, and starch is elaborated only in plants. But this is not, as it might seem to be, a fundamental distinction between the two, for many consider the animal mitochondria themselves to be plast-like and to act as such in the elaboration of secretions. The evidence for this, however, is not entirely conclusive and we must bear in mind, in all experimental work, the great importance of the homogeneous ground substance, or environment, in which the mitochondria are embedded. Apparent alterations may occur in the mitochondria which cannot be attributed to the mitochondria themselves, but only to changes in their environment. This is particularly true in the case of their refractive index. The mitochondria may stand out sharply in one cell and be quite invisible in another and yet be identical as far as their function is concerned in the two cells, for variations in the surrounding protoplasm may alone be responsible for the difference in appearance. There are thus two variables, the mitochondria and the protoplasm, either one of which may bring

about a visible difference in the living cell independently of the other, though in the majority of the cases they probably act together. So that it is very difficult to tell whether the mitochondria are active or passive agents.

The belief is gaining ground (Kingsbury, '12, p. 46; Mayer, Rathery and Schaeffer, '14, p. 619, and others) that mitochondria are concerned with respiration in animal cells; that is to say, with the taking up of oxygen. This conception is based upon the view that mitochondria chemically resemble phosphatids, it being thought by some that phosphatids outside the body are capable of auto-oxidation. It falls well in line with the very wide distribution of mitochondria as well as with the fundamental nature of the process of respiration. And of course, the same arguments apply to mitochondria in plant cells for plant cells also take up oxygen. It seems that, in this particular, there is no great difference between mitochondria in these two great groups of organisms.

Unfortunately it is not possible to transfer plant mitochondria to animal cells to see what they would do, or vice versa. We are working in the dark, but we have only ourselves to blame because we have not taken full advantage of certain lowly protozoans, like *Euglena viridis* and others, which have, confined in the scope of a single cell, all the properties which we are prone to consider distinctive of both plants and animals. They contain chlorophyll, produce paramylum, a carbohydrate resembling starch, and at the same time engulf, devour and digest certain still more minute organisms. The brief description of mitochondria-like material in *Euglena*, made by Ternetz ('12, p. 463), would serve as a point of departure. The planarian worm, *Convoluta roscoffensis*, which takes in algæ and lives in symbiotic relationship with them would repay further study. We have here an animal containing animal mitochondria with all their attributes which, after symbiotic relationship has been established, "ceases to take in solid food and depends entirely upon its vegetable partners" (Bayliss, '15, p. 295). Another opportunity is afforded by *Chlamydomyxa labyrinthuloides* which when at rest lives as a plant and when active like an animal (Parker and Haswell, '97, p. 49).



One point more. The close resemblance of mitochondria, amounting almost to identity in both plants and animals, may indicate a common ancestral form possessing mitochondria. It is a general assumption that plants antedate animals, and it seems significant that among the lower plants, and not in the lower animals, we find groups in which mitochondria have not been detected. I have particularly in mind the *Algæ*, for in this interesting group some forms contain mitochondria and others do not (M. and Mme. Moreau, '15, p. 730). The absence of mitochondria is interpreted in various ways. Some think that the large chloroplast takes over their function (Guilliermond, '13, p. 86) and it is possible that the mitochondrial substance may be present in an invisible diffuse condition. There is every reason to believe that the possession of formed mitochondria is an attribute even more primitive than the possession of a fully formed nucleus, which, unlike the mitochondria, has gradually assumed a more specialized character. Yeast plants in which the presence of a nucleus is debated (Macallum, '99, p. 67) unquestionably contain them (Janssens and Helsmortel, '13, p. 452), and there are indications that some bacteria also have mitochondrial material. This is beside the question, however, because the *Saccharomycetes* and *Schizomycetes* are probably degraded forms. We can look upon the ancient line of evolution as passing through forms closely resembling some of the *Algæ* of the present day which are devoid of mitochondria, into similar forms possessing mitochondria and which gradually acquired fully developed nuclei in addition. From these nucleated alga-like organisms containing mitochondria, the higher plants and the whole animal kingdom gradually evolved. They stood at the parting of the ways. The geological record, incomplete as it is, seems to indicate that the algæ are the oldest of plants; those of the present day being the lineal and comparatively unaltered descendants of the most ancient ones. It is surprising that mitochondria should persist without great modification through all the storm and stress of millions of years of evolution during which everything else changed except perhaps the primitive living homogeneous ground substance. As they were in the beginning so they are now, on the crest of both animal and plant

evolution, their form in *Vaucheria* (Rudolph, '12, Pl. 18, Fig. 9) being equally indistinguishable from that of the oak and of man. This paper shows how much alike they are everywhere. We are tempted to enquire whether the presence of mitochondria in conjunction with a nucleus made evolution possible. This may indeed be true if, as I have already mentioned (p. 223), they are concerned with protoplasmic respiration, which is perhaps the most fundamental of all vital manifestations. Certainly no consideration emphasizes their importance more.

#### SUMMARY.

This comparison of mitochondria in plant and animal cells brings to light a truly remarkable degree of similarity.

Their *reactions to fixatives, stains and supravital dyes* are almost identical. Similar fixatives preserve them, modify them and destroy them, in like manner, in both. Even the result of experimental errors in the technique is the same. Plant mitochondria react to the janus green test and stain with supravital dyes in substantially the same way as animal mitochondria, though it is certainly more difficult to obtain a good coloration.

Their *distribution* is almost universal. They occur in all plants with the exception of certain *Algæ*, *Bacteria* and *Myxomycetes*, and no animal has yet been discovered which does not contain them. In addition to this, similar and parallel variations occur in their arrangement in the several tissues and even in the individual cells of plants and animals. They must, therefore, be regarded as an integral, perhaps essential, constituent of living matter.

Their *morphology* is identical in plants and in animals. They assume no forms in the one, which are not present in the other. They undergo similar variations in size and shape in different tissues and in different cells in both. If it were possible to view mitochondria dissociated from their environment, it would be impossible to decide whether they came from plant or animal tissues, provided that they did not contain starch, pigment or some other easily recognizable substance, to serve as a clue.

We have every reason to suppose that their chemical *composition* is much the same in both plants and animals, but here our

knowledge is for the most part supposition and inference, since direct chemical analyses are obviously out of the question. Their composition, as indicated by solubility with respect to acetic acid, heat and other agents, is certainly subject to similar variations in both.

Their *physiology* is obscure; but their wide occurrence in all protoplasm and their general similarity in all places must mean something. It may mean that, in addition to certain specific functions like the production of chlorophyll, they all have a common duty or part to play in some fundamental vital manifestation like protoplasmic respiration.

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